REMARKS

Status of the Claims

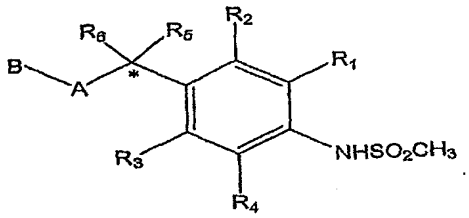
Claims 15-17 and 20-29 are pending. Claim 15 is the only independent claim. In this Reply, claim 15 has been amended and claims 18-19 have been canceled. Claims 20-21 are withdrawn pursuant to the species election requirement set forth in the Office Action dated April 3, 2009. No new matter has been added.

Applicants thank the Examiner for withdrawing the restriction requirement set forth in the Office Action dated April 3, 2009. However, Applicants respectfully request the Examiner to reconsider and withdraw the current rejection in view of the foregoing amendments and the following remarks.

Rejection Under 35 U.S.C. § 103

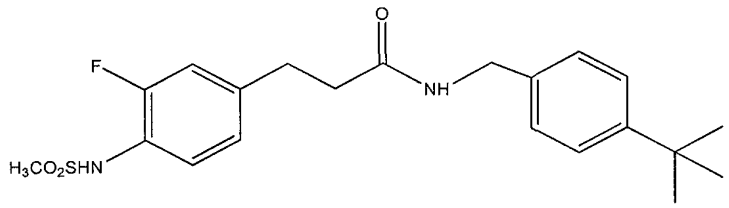
The rejection of claims 15-17 and 22-29 under 35 U.S.C. § 103(a) over WO 2002/016318 ("Suh et al.") is respectfully traversed.

The presently claimed compounds of independent claim 15 are novel and inventive vanilloid receptor (VR1) antagonists. They can unexpectedly exhibit excellent affinity for VR1 and consequently excellent analgesic activity. The compounds of independent claim 15, as amended, correspond to formula (I) or a pharmaceutically acceptable salt or isomer thereof. A, B, and R_1-R_6 are as defined above. In particular, A is CONH, NHCO, or NHC(=O)NH. Also, R_5 and R_6 are not hydrogen atoms simultaneously and the asteric mark * indicates a chiral carbon atom.

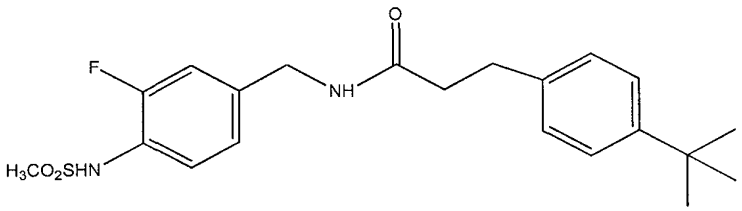


(I)

In contrast, Suh et al. discloses thiourea derivatives as modulators for the vanilloid receptor. In the Examples, Suh et al. discloses the following compounds:



Example 168



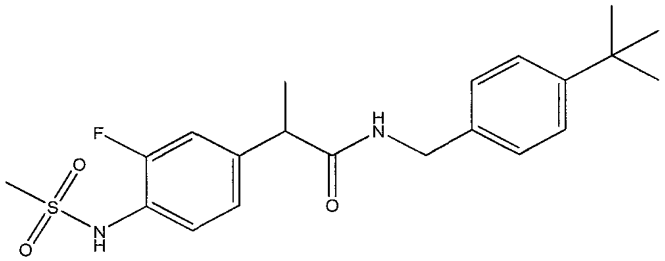
Example 193

The presently claimed compounds are non-obvious over Suh et al. because Suh et al. does not disclose or suggest each and every element of independent claim 15. In particular, Suh et al. does not disclose or suggest the presently claimed compounds where R_5 and R_6 are not hydrogen atoms simultaneously and include the presently recited chiral carbon atom represented by the asterisk mark *. Thus, the presently claimed compounds are not *prima facie* obvious over Suh et al.

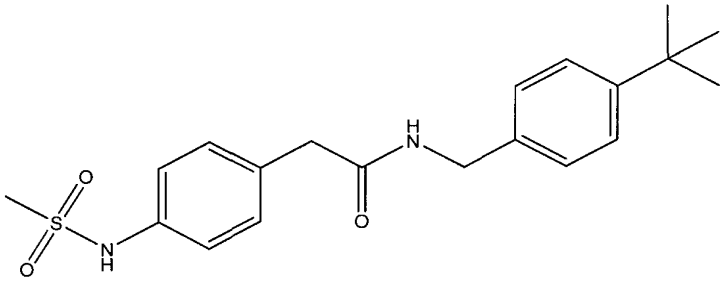
Furthermore, even if the presently claimed compounds were *prima facie* obvious over Suh et al., the presently claimed compounds can unexpectedly exhibit excellent affinity for VR1, and therefore, can unexpectedly exhibit excellent analgesic activity. These unexpected results rebut any *prima facie* case of obviousness over Suh et al.

The compound N-(4-tert-butylbenzyl)-2-[3-fluoro-4-(methylsulfonylamino)phenyl]propionamide ("Compound A") exhibits excellent

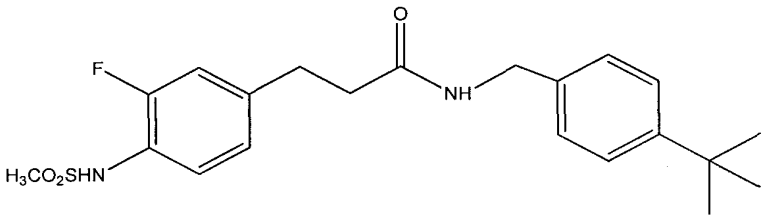
affinity to VR1 as compared to N-(4-tert-butylbenzyl)-2-[4-(methylsulfonylamino)phenyl]acetamide ("Compound B"). Compound A is one of the presently claimed compounds and disclosed in Example 1 on page 36 of the specification. In contrast, Compound B could be derived from formula (I) of Suh et al. by selecting the appropriate substituents. Importantly, Compound B is very structurally similar to the compound of Example 168 of Suh et al. Likewise, Compound A is very structurally similar to Suh et al.'s Compound B and Example 168 compound. Compound A, Compound B, and the Example 168 compound are depicted below.



Compound A



Compound B



Example 168

In two experimental assays, Compound A demonstrated significantly better affinity for VR1 than Compound B. The assays are described in the Appendix attached to this Reply. Indeed, Compound A demonstrated affinity for VR1 at least 20 times greater than Compound B:

Compound	K_i (Assay #1)	K_i (Assay #2)
A	19.1 nM	44.5 nM
B	> 1000 nM	> 1000 nM

These assays demonstrate that minor structural variations to the cited compounds of Suh et al. unexpectedly enhance their pharmacological activity. These unexpected results effectively rebut any *prima facie* case of obviousness over Suh et al.

Therefore, for at least the reasons discussed above, withdrawal of the obviousness rejection over Suh et al. is respectfully requested.

Conclusion

The application is respectfully submitted to be in condition for allowance, and prompt, favorable action thereon is earnestly solicited.

If there are any questions regarding this Reply or the application in general, a telephone call to the undersigned at (202) 624-2845 would be appreciated since this should expedite the examination of the application.

If necessary to effect a timely response, this paper should be considered as a petition for an Extension of Time sufficient to effect a timely response, and please charge any deficiency in fees or credit any overpayments to Deposit Account No. 05-1323 (Docket # 029310.57239US).

Respectfully submitted,

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APPENDIX

Assay #1:

The agonistic or antagonistic action of the substances to be investigated on the vanilloid receptor 1 (VR1/TRPV1) of the rat species may be determined by the following assay. According to this assay, the influx of Ca^{2+} through the receptor channel is quantified with the assistance of a Ca^{2+}-sensitive dye (type Fluo-4, Molecular Probes Europe BV, Leiden, Netherlands) in a Fluorescent Imaging Plate Reader (FLIPR, Molecular Devices, Sunnyvale, USA).

Method:

Complete medium: 50 mL HAMS F12 Nutrient Mixture (Gibco Invitrogen GmbH, Karlsruhe, Germany) with 10 vol.% FCS (foetal calf serum, Gibco Invitrogen GmbH, Karlsruhe, Germany, heat-inactivated); 2 mM L-glutamine (Sigma, Munich, Germany); 1 wt.% AA solution (antibiotic/antimycotic solution, PAA, Pasching, Austria) and 25 ng/mL NGF medium (2.5 S, Gibco Invitrogen GmbH, Karlsruhe, Germany); Cell culture plate: poly-D-lysine coated, black 96 well plates with a clear bottom (96 well black/clear plate, BD Biosciences, Heidelberg, Germany) are additionally coated with laminin (Gibco Invitrogen GmbH, Karlsruhe, Germany), by diluting laminin to a concentration of 100 µg/mL with PBS (Ca-Mg-free PBS, Gibco Invitrogen GmbH, Karlsruhe, Germany). Aliquots with a concentration of 100 µg/mL of laminin are taken and stored at -20°C. The aliquots are diluted with PBS in a 1:10 ratio to 10 µg/ml. of laminin and a 50 µL portion is in each case pipetted into a well of the cell culture-plate. The cell culture-plates are incubated at 37°C for at least two hours, the supernatant solution is aspirated and the wells are in each case washed twice with PBS. The coated cell culture-plates are stored with supernatant PBS, which is not removed until just before application of the cells.

Preparation of the cells:

The spinal column is removed from decapitated rats and is placed directly in cold, i.e. located in an ice bath, HBSS buffer (Hank's buffered saline solution, Gibco Invitrogen GmbH, Karlsruhe, Germany) combined with 1 vol.% (percent by volume) of an AA solution (antibiotic/antimycotic solution, PAA, Pasching, Austria). The spinal column is cut open longitudinally and removed together with fasciae from the spinal canal. The dorsal root ganglia (DRGs) are then removed and in turn stored in cold HBSS buffer combined with 1 vol.% of an AA solution. The DRGs, from which all traces of blood and spinal nerves have been removed, are in each case transferred into 500 µL of cold collagenase type 2 (PAA, Pasching, Austria) and incubated for 35 minutes at 37°C. After addition of 2.5 vol.% of trypsin (PAA, Pasching, Austria), incubation is continued for a further 10 minutes at 37°C. Once incubation is complete, the enzyme solution is carefully removed by pipette and the DRGs, which are left behind, are in each case combined with 500 µL of complete medium.

The DRGs are in each case repeatedly suspended, drawn by means of a syringe through no. 1, no. 12 and no. 16 cannulas and transferred into 50 mL Falcon microtubes and each tube is made up to 15 mL with complete medium. The contents of each Falcon microtube are in each case filtered through a 70 µm Falcon filter insert and centrifuged for 10 minutes at 1200 revolutions and room temperature. The resultant pellet is in each case resuspended in 250 µL of complete medium and the cell count determined.

The number of cells in the suspension is adjusted to 3×10^5 per mL and a 150 µL portion of this suspension is in each case placed in a well of the cell culture plate which has been coated as described above. The plates are placed in an incubator at 37°C, 5 vol.% CO2 and 95% relative atmospheric humidity for two to three days.

The cells are then loaded with 2 µM Fluo-4 and 0.01 vol.% Pluronic F127 (Molecular Probes Europe BV, Leiden, Netherlands) in HBSS buffer (Hank's buffered saline solution, Gibco Invitrogen GmbH, Karlsruhe, Germany) for 30 min at

37°C, washed 3 x with HBSS buffer and, after a further 15 minutes' incubation at room temperature, used for Ca^{2+} measurement in the FLIPR assay. Ca^{2+}-dependent fluorescence is here measured before and after the addition of substances (λex = 488 nm, λem = 540 nm). Quantification proceeds by measuring the highest fluorescence intensity (FC, fluorescence counts) over time.

FLIPR assay:

The FLIPR protocol comprises 2 additions of substance. The compounds to be tested (10 µM) are firstly pipetted onto the cells and Ca^2 influx is compared with the control (capsaicin 10 µM). This provides the percentage activation relative to the Ca^{2+} signal after addition of 10 µM of capsaicin (CP). After 5 minutes incubation, 100 nM of capsaicin are added and the influx of Ca^{2+} is again determined.

Desensitising agonists and antagonists result in suppression of Ca^{2+} influx. The percentage inhibition in comparison with the maximum achievable inhibition with 10 µM capsaicin is calculated.

The FLIPR protocol can also be carried out by using the compounds to be tested in a concentration of 1 µM. In this case capsaicin is also used in a concentration of 1 µM and the calculation is based on the maximum achievable inhibition with 1 µM capsaicin.

Triplicate determinations (n=3) are performed and these are repeated in at least 3 independent experiments (N=4).

On the basis of the percentage displacement by different concentrations of the compounds to be tested of the general formula I, IC_{50} inhibition concentrations which bring about 50% displacement of capsaicin were calculated. K_i values for the test substances were obtained by conversion using the Cheng-Prusoff equation (Cheng, Prusoff; Biochem. Pharmacopoeia. 22, 3099-3108, 1973).

Assay #2:

The agonistic or antagonistic action of the substances to be investigated on the vanilloid receptor (VR1) may also be determined with the following assay. According to this assay, the influx of Ca^{2+} through the channel is quantified with the assistance of a Ca^{2+}-sensitive dye (type Fluo-4, Molecular Probes Europe BV, Leiden, Netherlands) in a Fluorescent Imaging Plate Reader (FLIPR, Molecular Devices, Sunnyvale, USA).

Method:

Chinese hamster ovary cells (CHO K1 cells, European Collection of Cell Cultures (ECACC), Great Britain) are stably transfected with the VR1 gene. For functional investigations, these cells are plated out onto poly-D-lysine-coated, black 96 well plates with a clear bottom (BD Biosciences, Heidelberg, Germany) at a density of 25,000 cells/well. The cells are incubated overnight at 37°C and 5% CO_2 in a culture medium (Ham's Nutrient Mixture F12, 10 vol.% FCS (foetal calf serum), 18 µg/mL L-proline). On the following day, the cells are incubated with Fluo-4 (Fluo-4 2 µM, Pluronic F127 0.01 vol.%, Molecular Probes in HBSS (Hank's buffered saline solution), Gibco Invitrogen GmbH, Karlsruhe, Germany) for 30 minutes at 37°C. The plates are then washed 3 times with HBSS buffer and, after a further 15 minutes incubation at room temperature, used for Ca^{2+} measurement in the FLIPR. Ca^{2+}-dependent fluorescence is here measured before and after addition of the substances to be investigated (wavelength λ_{ex} = 488 nm, λ_{em} = 540 nm). Quantification proceeds by measuring the highest fluorescence intensity (FC, fluorescence counts) over time.

FLIPR assay:

The FLIPR protocol comprises 2 additions of substance. The substances to be tested (10 µM) are firstly pipetted onto the cells and Ca^{2+} influx is compared with the control (capsaicin 10 µM) (% activation relative to the Ca^{2+} signal after addition of 10 µM of capsaicin). After 5 minutes incubation, 100 nM of capsaicin are added and the influx of Ca^{2+} is again determined.

Desensitising agonists and antagonists resulted in suppression of Ca^{2+} influx. The percentage inhibition in comparison with the maximum achievable inhibition with 10 µM capsaicin is calculated.

The FLIPR protocol can also be carried out by using the compounds to be tested in a concentration of 1 µM. In this case capsaicin is also used in a concentration of 1 µM and the calculation is based on the maximum achievable inhibition with 1 µM capsaicin.

On the basis of the percentage displacement by different concentrations of the compounds to be tested of the general formula I, IC_{50} inhibition concentrations which bring about 50% displacement of capsaicin were calculated. K_i values for the test substances were obtained by conversion using the Cheng-Prusoff equation (Cheng, Prusoff; Biochem. Pharmacopoeia. 22, 3099-3108, 1973).